

# TRhizo-urbanMicrobiome

## Microbiome Assembly Piecewise SEM

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## Load Packages

## Load Data

```
## Microbiome pSEM data
microbiome.pSEM.data <- read_rds(
  file = "data/microbiome_pSEM_data.rds"
) %>%
  select(
    Population, Root_Weighted_UniFrac_PCoA_1, Root_Weighted_UniFrac_PCoA_2,
    Soil_Weighted_UniFrac_PCoA_1, Soil_Weighted_UniFrac_PCoA_2,
    Distance:Mean_ISC, Leaf_Total_Carbon, Leaf_Delta_13C, Soil_Total_Carbon, Soil_Delta_13C,
    Leaf_Total_Nitrogen, Leaf_Delta_15N, Soil_Total_Nitrogen, Soil_Delta_15N
  )
```

# Piecewise SEM

## pSEM Specification

```
microbiome.pSEM.model.list <- psem(  
  ## Urbanization metrics-----  
  lm(  
    Mean_ISC ~ Distance,  
    data = microbiome.pSEM.data  
  ),  
  lm(  
    Human_Influence_Index ~ Distance,  
    data = microbiome.pSEM.data  
  ),  
  
  # Correlations  
  Mean_ISC %~~% Human_Influence_Index,  
  
  ## Soil Carbon-----  
  lm(  
    Soil_Total_Carbon ~ Mean_ISC + Human_Influence_Index,  
    data = microbiome.pSEM.data  
  ),  
  lm(  
    Soil_Delta_13C ~ Mean_ISC + Human_Influence_Index + Distance, # + Distance  
    data = microbiome.pSEM.data  
  ),  
  
  # Correlations  
  Soil_Total_Carbon %~~% Soil_Delta_13C,  
  Soil_Total_Carbon %~~% Soil_Total_Nitrogen,  
  
  ## Soil Nitrogen-----  
  lm(  
    Soil_Total_Nitrogen ~ Mean_ISC + Human_Influence_Index + Distance, # + Distance  
    data = microbiome.pSEM.data  
  ),  
  lm(  
    Soil_Delta_15N ~ Mean_ISC + Human_Influence_Index,  
    data = microbiome.pSEM.data  
  ),  
  
  # Correlations  
  Soil_Total_Nitrogen %~~% Soil_Delta_15N,  
  Soil_Total_Nitrogen %~~% Soil_Delta_13C, # Added following d-sep test  
  
  ## Leaf Carbon-----  
  lm(  
    Leaf_Total_Carbon ~ Mean_ISC + Human_Influence_Index  
      + Root_Weighted_UniFrac_PCoA_1 + Root_Weighted_UniFrac_PCoA_2 + Soil_Delta_15N, # + Soil_Delta_13C
```

```

    data = microbiome.pSEM.data
),
lm(
  Leaf_Delta_13C ~ Mean_ISC + Human_Influence_Index
    + Root_Weighted_UniFrac_PCoA_1 + Root_Weighted_UniFrac_PCoA_2,
  data = microbiome.pSEM.data
),

# Correlations
Leaf_Total_Carbon %~~% Leaf_Delta_13C,
Leaf_Total_Carbon %~~% Leaf_Total_Nitrogen,

## Leaf Nitrogen-----
lm(
  Leaf_Total_Nitrogen ~ Mean_ISC + Human_Influence_Index
    + Root_Weighted_UniFrac_PCoA_1 + Root_Weighted_UniFrac_PCoA_2
    + Soil_Total_Nitrogen + Soil_Delta_15N,
  data = microbiome.pSEM.data
),
lm(
  Leaf_Delta_15N ~ Mean_ISC + Human_Influence_Index
    + Root_Weighted_UniFrac_PCoA_1 + Root_Weighted_UniFrac_PCoA_2
    + Soil_Total_Nitrogen + Soil_Delta_15N,
  data = microbiome.pSEM.data
),

# Correlations
Leaf_Total_Nitrogen %~~% Leaf_Delta_15N,

## Soil microbiome-----
lm(
  Soil_Weighted_UniFrac_PCoA_1 ~ Soil_Total_Carbon + Soil_Delta_13C
    + Soil_Total_Nitrogen + Soil_Delta_15N + Mean_ISC + Human_Influence_Index,
  data = microbiome.pSEM.data
),
lm(
  Soil_Weighted_UniFrac_PCoA_2 ~ Soil_Total_Carbon + Soil_Delta_13C
    + Soil_Total_Nitrogen + Soil_Delta_15N + Mean_ISC + Human_Influence_Index,
  data = microbiome.pSEM.data
),

# Correlations
Soil_Weighted_UniFrac_PCoA_1 %~~% Soil_Weighted_UniFrac_PCoA_2,

## Root Microbiome-----
lm(
  Root_Weighted_UniFrac_PCoA_1 ~ Soil_Weighted_UniFrac_PCoA_1 + Soil_Weighted_UniFrac_PCoA_2
    + Soil_Total_Carbon + Soil_Delta_13C + Soil_Total_Nitrogen + Soil_Delta_15N
    + Mean_ISC + Human_Influence_Index,
  data = microbiome.pSEM.data
)

```

```

),
lm(
  Root_Weighted_UniFrac_PCoA_2 ~ Soil_Weighted_UniFrac_PCoA_1 + Soil_Weighted_UniFrac_PCoA_2
  + Soil_Total_Carbon + Soil_Delta_13C + Soil_Total_Nitrogen + Soil_Delta_15N
  + Mean_ISC + Human_Influence_Index,
  data = microbiome.pSEM.data
),

# Correlations
Root_Weighted_UniFrac_PCoA_1 %~~% Root_Weighted_UniFrac_PCoA_2
)

```

```

## Fit component structural equation
structural.equation.LM <- lm(
  Y ~ X,
  data = microbiome.pSEM.data
)
# Change the model formula for each structural equation in the pSEM list

## Visual assessment of model fit
check_model(structural.equation.LM)

```

## pSEM Evaluation

```
## pSEM summary
microbiome.pSEM.summary <- summary(
  microbiome.pSEM.model.list,
  conserve = TRUE
)

## Test of directed separation
microbiome.pSEM.summary.directed.separation <- dSep(
  microbiome.pSEM.model.list,
  conserve = TRUE
)

## Get Fisher's C statistics of model fit
fisherC(microbiome.pSEM.model.list, conserve = TRUE)
# Fisher's C = 77.027, df = 68, P = 0.212

## R-squared values for endogenous variables
microbiome.pSEM.R.squared <- rsquared(
  microbiome.pSEM.model.list
)

## Standardized coefficients
microbiome.pSEM.path.coefficients <- coefs(
  microbiome.pSEM.model.list,
  standardize = "scale"
)
```

## pSEM Path Coefficients

Table 1: Path coefficients for each causal and correlational pathway in the microbiome assembly piecewise SEM. Global model fit: Fisher's  $C = 77.027$ ,  $df = 68$ ,  $P = 0.212$ .  $R^2$  values for endogenous variables: Mean ISC = 0.624, Human Influence Index = 0.627, Soil Total C = 0.218, Soil 13C = 0.378, Leaf Total C = 0.249, Leaf 13C = 0.191, Soil Total N = 0.192, Soil 15N = 0.335, Leaf Total N = 0.412, Leaf 15N = 0.236, Soil PCoA 1 = 0.184, Soil PCoA 2 = 0.623, Root PCoA 1 = 0.347, Root PCoA 2 = 0.358.

Response	Predictor	Estimate	Std.Error	Std.Estimate	P.Value
Mean_ISC	Distance	-1.491	0.2015	-0.790	0.000
Human_Influence_Index	Distance	-0.528	0.0709	-0.792	0.000
~~Mean_ISC	~~Human_Influence_Index	0.240	-	0.240	0.085
Soil_Total_Carbon	Mean_ISC	0.019	0.0145	0.287	0.209
Soil_Total_Carbon	Human_Influence_Index	-0.115	0.041	-0.626	0.009
Soil_Delta_13C	Mean_ISC	0.010	0.0479	0.049	0.839
Soil_Delta_13C	Human_Influence_Index	-0.011	0.1362	-0.019	0.938
Soil_Delta_13C	Distance	0.243	0.1035	0.638	0.026
~~Soil_Total_Carbon	~~Soil_Delta_13C	0.398	-	0.398	0.010
~~Soil_Total_Carbon	~~Soil_Total_Nitrogen	0.366	-	0.366	0.017
Soil_Total_Nitrogen	Mean_ISC	0.000	0.001	-0.070	0.799
Soil_Total_Nitrogen	Human_Influence_Index	-0.003	0.0028	-0.304	0.272
Soil_Total_Nitrogen	Distance	-0.005	0.0021	-0.686	0.034
Soil_Delta_15N	Mean_ISC	0.012	0.0106	0.237	0.260
Soil_Delta_15N	Human_Influence_Index	0.056	0.0299	0.386	0.071
~~Soil_Total_Nitrogen	~~Soil_Delta_15N	0.349	-	0.349	0.021
~~Soil_Total_Nitrogen	~~Soil_Delta_13C	-0.475	-	-0.475	0.002
Leaf_Total_Carbon	Mean_ISC	0.023	0.0131	0.413	0.091
Leaf_Total_Carbon	Human_Influence_Index	-0.077	0.0406	-0.487	0.069
Leaf_Total_Carbon	Root_Weighted_UniFrac_PCoA_1	5.762	14.1125	0.071	0.686
Leaf_Total_Carbon	Root_Weighted_UniFrac_PCoA_2	0.025	7.3524	0.001	0.997
Leaf_Total_Carbon	Soil_Delta_15N	0.462	0.2147	0.426	0.040
Leaf_Delta_13C	Mean_ISC	-0.008	0.008	-0.229	0.341
Leaf_Delta_13C	Human_Influence_Index	0.003	0.0239	0.027	0.916
Leaf_Delta_13C	Root_Weighted_UniFrac_PCoA_1	-19.326	8.7227	-0.390	0.034
Leaf_Delta_13C	Root_Weighted_UniFrac_PCoA_2	5.302	4.5542	0.221	0.254
~~Leaf_Total_Carbon	~~Leaf_Delta_13C	0.031	-	0.031	0.430
~~Leaf_Total_Carbon	~~Leaf_Total_Nitrogen	0.421	-	0.421	0.006
Leaf_Total_Nitrogen	Mean_ISC	0.007	0.0043	0.361	0.104
Leaf_Total_Nitrogen	Human_Influence_Index	-0.018	0.0131	-0.322	0.179
Leaf_Total_Nitrogen	Root_Weighted_UniFrac_PCoA_1	3.640	4.8047	0.125	0.455
Leaf_Total_Nitrogen	Root_Weighted_UniFrac_PCoA_2	1.822	2.3795	0.129	0.450
Leaf_Total_Nitrogen	Soil_Total_Nitrogen	0.247	0.9488	0.045	0.797
Leaf_Total_Nitrogen	Soil_Delta_15N	0.211	0.0754	0.544	0.009
Leaf_Delta_15N	Mean_ISC	0.004	0.0047	0.209	0.400
Leaf_Delta_15N	Human_Influence_Index	-0.013	0.0146	-0.230	0.395
Leaf_Delta_15N	Root_Weighted_UniFrac_PCoA_1	7.547	5.3512	0.265	0.170
Leaf_Delta_15N	Root_Weighted_UniFrac_PCoA_2	-4.976	2.6501	-0.361	0.071
Leaf_Delta_15N	Soil_Total_Nitrogen	0.547	1.0567	0.102	0.609
Leaf_Delta_15N	Soil_Delta_15N	0.088	0.084	0.233	0.302
~~Leaf_Total_Nitrogen	~~Leaf_Delta_15N	0.270	-	0.270	0.061
Soil_Weighted_UniFrac_PCoA_1	Soil_Total_Carbon	0.003	0.0038	0.222	0.479
Soil_Weighted_UniFrac_PCoA_1	Soil_Delta_13C	0.000	0.0013	-0.083	0.810
Soil_Weighted_UniFrac_PCoA_1	Soil_Total_Nitrogen	0.015	0.0761	0.070	0.844
Soil_Weighted_UniFrac_PCoA_1	Soil_Delta_15N	0.004	0.0037	0.273	0.267
Soil_Weighted_UniFrac_PCoA_1	Mean_ISC	0.000	2e-04	-0.409	0.146
Soil_Weighted_UniFrac_PCoA_1	Human_Influence_Index	0.001	6e-04	0.363	0.216
Soil_Weighted_UniFrac_PCoA_2	Soil_Total_Carbon	0.010	0.0025	0.813	0.001
Soil_Weighted_UniFrac_PCoA_2	Soil_Delta_13C	-0.001	9e-04	-0.318	0.182

Soil_Weighted_UniFrac_PCoA_2	Soil_Total_Nitrogen	-0.044	0.0515	-0.205	0.398
Soil_Weighted_UniFrac_PCoA_2	Soil_Delta_15N	-0.004	0.0025	-0.251	0.137
Soil_Weighted_UniFrac_PCoA_2	Mean_ISC	0.000	1e-04	-0.099	0.598
Soil_Weighted_UniFrac_PCoA_2	Human_Influence_Index	0.000	4e-04	-0.032	0.869
~~Soil_Weighted_UniFrac_PCoA_1	~~Soil_Weighted_UniFrac_PCoA_2	-0.108	-	-0.108	0.271
Root_Weighted_UniFrac_PCoA_1	Soil_Weighted_UniFrac_PCoA_1	0.383	0.1528	0.442	0.019
Root_Weighted_UniFrac_PCoA_1	Soil_Weighted_UniFrac_PCoA_2	0.002	0.2258	0.003	0.991
Root_Weighted_UniFrac_PCoA_1	Soil_Total_Carbon	0.000	0.0038	0.028	0.939
Root_Weighted_UniFrac_PCoA_1	Soil_Delta_13C	0.001	0.0011	0.200	0.549
Root_Weighted_UniFrac_PCoA_1	Soil_Total_Nitrogen	0.076	0.062	0.405	0.232
Root_Weighted_UniFrac_PCoA_1	Soil_Delta_15N	-0.005	0.0031	-0.370	0.130
Root_Weighted_UniFrac_PCoA_1	Mean_ISC	0.000	2e-04	0.043	0.872
Root_Weighted_UniFrac_PCoA_1	Human_Influence_Index	0.000	5e-04	0.077	0.781
Root_Weighted_UniFrac_PCoA_2	Soil_Weighted_UniFrac_PCoA_1	0.039	0.3132	0.022	0.901
Root_Weighted_UniFrac_PCoA_2	Soil_Weighted_UniFrac_PCoA_2	0.653	0.463	0.363	0.170
Root_Weighted_UniFrac_PCoA_2	Soil_Total_Carbon	0.004	0.0078	0.181	0.618
Root_Weighted_UniFrac_PCoA_2	Soil_Delta_13C	0.000	0.0023	0.060	0.856
Root_Weighted_UniFrac_PCoA_2	Soil_Total_Nitrogen	0.017	0.1271	0.043	0.896
Root_Weighted_UniFrac_PCoA_2	Soil_Delta_15N	0.002	0.0065	0.091	0.703
Root_Weighted_UniFrac_PCoA_2	Mean_ISC	0.000	4e-04	0.005	0.985
Root_Weighted_UniFrac_PCoA_2	Human_Influence_Index	-0.001	0.0011	-0.168	0.541
~~Root_Weighted_UniFrac_PCoA_1	~~Root_Weighted_UniFrac_PCoA_2	0.294	-	0.294	0.046



## R Session Information

Table 2: Packages required for data management and analysis.

Package	Loaded Version	Date
dplyr	1.1.4	2023-11-17
forcats	1.0.0	2023-01-29
ggplot2	3.5.1	2024-04-23
kableExtra	1.4.0	2024-01-24
knitr	1.46	2024-04-06
lubridate	1.9.3	2023-09-27
performance	0.11.0	2024-03-22
piecewiseSEM	2.3.0	2023-03-04
purrr	1.0.2	2023-08-10
readr	2.1.5	2024-01-10
stringr	1.5.1	2023-11-14
tibble	3.2.1	2023-03-20
tidyr	1.3.1	2024-01-24
tidyverse	2.0.0	2023-02-22